REMARKS/ARGUMENTS

In addition to the claims which were canceled during previous prosecution, please cancel claims 52-61 and 64-69, without prejudice or disclaimer. Thus, claims 1-44, 52-61 and 64-69 are canceled. Applicants reserve the right to pursue the subject matter of the canceled claims during later prosecution. New claims 71 and 72 express the substance of claim 45 in alternative wording. Claims 45-51, 62, 63 and 70-72 are currently under consideration. The claims under consideration are directed to methods for producing a coated material, comprising preparing a coating agent by methods comprising treating yeast with enzymes, acid and water.

The recitation in amended independent claims 45 and 70, and in new claims 71 and 72, that the yeast have not been treated with alkali is fully supported in the specification. See, for example, page 5, lines 18-24: "An excellent enteric coating ... was unexpectedly obtained using only acidic aqueous solution in varying concentrations, unlike conventional conditions for treating yeast with both alkali and acid treatments ..." The recitation of an air-tight (air-impermeable) coating (film) is fully supported in the specification, *e.g.*, at page 13, lines 23-25 ["Coating layers (films) comprising the coating of the present invention have an extremely low oxygen or other gas permeability and moisture."]. The extremely low oxygen or other gas impermeability suggests that the coating is effectively airtight.

The rejection of claims 59-61 and 65-67 under 35 U.S.C. §112 is rendered moot by the cancellation of those claims.

Contrary to the allegation of the Examiner, USP 5,521,089 ("Ishiguro") does not render the instant claims obvious.

Preparation of the coating agent without exposing the yeast to alkali

Instant independent claims 45 and 70-72 recite methods for producing a coating agent or a coated material, comprising using cell wall residue from yeast which has been treated with enzymes, acid and water, but which has not been treated with alkali, wherein the coating agent can form a continuous, air-tight film on a solid material, to form a coating thereon. By contrast, Ishiguro discloses a method for producing yeast

microcapsules, which are suitable for encapsulating hydrophobic liquids. In Ishiguro's methods, yeast are treated under a variety of conditions, including treatment with enzymes and treatment with alkali; however, Ishiguro does not recognize that it is important *not* to treat the yeast with alkali, even when using the enzymatic methods. Furthermore, Ishiguro does not teach a method of preparing a coating agent that can form a continuous, air-tight film (coating) on a solid material. The coating would necessarily be different since the conditions of preparation differ. The reference provides no motivation to modify its method to require that the yeast is not treated with alkali, or to produce a coating agent that can form a continuous, air-tight film (coating) on a solid material.

If anything, Ishiguro suggests away from preparing its microcapsules in the absence of alkali. The reference compares methods in which yeast are treated with alkali (Examples 1, 2, 3, 4 and 5) to methods in which the yeast are not treated with alkali (*e.g.*, Comparative Example 1). In Comparative Example 1, the patent specifies that the yeast are prepared "without subjecting the dispersion [of the yeast cell residue] to the alkali treatment" (col. 6, line 6). Comparisons of properties of microcapsules provided with or without alkali are shown in Tables 1 and 2 of the patent. Ishiguro reports that MC prepared by methods comprising treatment with alkali provide superior properties (*e.g.*, for use in preparing pressure-sensitive copying paper) compared to methods in which the yeast are not treated with alkali.

The present inventors have recognized that coating agents prepared by the methods of the invention, in the absence of treatment with alkali, exhibit unexpectedly superior properties (*e.g.*, for coating food products or pharmaceutical products). See, *e.g.*, the discussion of Comparative Example 1 in the specification at pages 30 and 31, which indicates that the method of the invention is superior to the method of Comparative Example 1 (which is a "combined acid and *alkali* treatment"). The latter, comparative, method provides (a) a lower yield of yeast cell wall fraction, which is so viscous that the coating process is slowed, because the coating process must be carried out in dilute concentration or at a low feeding rate; and (b) a product that, because of its prolonged treatment with alkali, is likely to contain the toxic product, lysinoalanine, which is unsuitable for food products or pharmaceutical products.

The first attached Declaration[1] provides further evidence that coating agents prepared in the absence of alkali exhibit superior properties (coating properties) compared to microcapsules prepared by the methods of Ishiguro in which the yeast are subjected to alkali treatment. This Declaration describes microcapsules prepared by either of two methods exemplified by Ishiguro: (1) microcapsules produced by the method of Example 3 of the reference (enzyme digestion, followed by an alkali treatment - sample A), and (2) microcapsules produced by the method of Example 1 of the reference (treatment with alkali - sample B). The Declaration also describes a coating agent made by a method of the instant invention, using the protocol of Example 6 (yeast autolysis (an enzyme treatment), followed by an acid treatment).

Each of the three preparations is used to generate a coating film. The properties of these films are shown in Table 1 and in Figures 1-3 of the Declaration. The film generated from material produced by the method of the invention (in the absence of alkali) exhibits properties which are advantageous for coating and protecting materials, such as tablets. The film is continuous and uniform, and exhibits considerable tensile strength. By contrast, the films generated from material produced by the methods of Ishiguro involving alkali treatment have cracks or voids and are so fragile their tensile strength cannot even be measured. Clearly, the coating agent produced by the method of the invention exhibits superior properties.

The second attached Declaration[1] provides still further evidence that coating agents prepared in the absence of alkali exhibit superior properties (dissolution properties) compared to microcapsules prepared by the method of Ishiguro in which the yeast are subjected to alkali treatment. This Declaration shows a comparison of dissolution profiles of tablets containing the pharmaceutical, acetaminophen (AAP), in which the pharmaceutical is coated either with a coating agent of the invention or with a coating agent prepared by a method of Ishiguro. A coating agent of the invention is prepared by yeast autolysis (an enzyme treatment) followed by an acid treatment, to produce "AYC" preparations (see Example 6 in the present application). A coating agent according to the method of Ishiguro is prepared by autolysis followed by alkali treatment

[1] The attached two inventor Declarations are not yet executed. Executed copies will be provided to the Examiner shortly.

(see Example 1 in the reference). The release of the AAP from the particles is determined, using a paddle method. Table 2 of this Declaration shows that, with coatings of the invention, there is a considerable lag before dissolution begins, and the dissolution follows a sigmoidal release profile. By contrast, with a coating prepared by alkali treatment, the dissolution (in water) begins immediately, and follows a gradual release (sustained release) profile. The sigmoidal pattern of dissolution is shown in Figure 1 of this Declaration.

Some advantages of coatings which allow the controlled release of substances that they encapsulate (*e.g.*, by controlling dissolution of the coated tablet, wherein a lag time is followed by rapid dissolution) are disclosed in the instant specification. See, *e.g.*, page 1, lines 9-10 ("coated particles ... with the function of controlling dissolution time"); page 4, lines 13-15 ("a coating agent with better enteric properties, allowing the time at which dissolution begins to be controlled"); Example 5; and page 32, section (2). The dissolution profile of tablets prepared by a method of the invention is advantageous, for example for time-controlled release in drug delivery systems (*e.g.*, for enteric coatings), or for masking bitter taste or offensive smell of an encapsulated substance (see, *e.g.*, Example 1, particularly Fig. 1 and page 19, lines 2-7; and Example 10, particularly Fig. 8 and page 28, lines 13-20).

The instant specification also discloses further advantageous properties of the inventive coating agents: the coating agents are excellent enteric coating agents that are capable of encapsulating greater amounts of a substance without compromising the function of protecting the encapsulated substance; the time at which the coated tablet begins to dissolve (*e.g.*, following administration to a subject) can be controlled; the coatings have a non-sticky finish, and they exhibit an extremely low permeability to oxygen or other gasses and to moisture. See, *e.g.*, the specification at page 5, lines 7-12; page 12, lines 11-17; and page 13, line 16 to page 14, line 1.

Claims 63 and 70

Claims 63 and 70 are further distinguished from the methods of Ishiguro, in that these claims recite steps which exclude the presence of a hydrophobic liquid within the coating material, which is clearly taught by Ishiguro.

Claim 63 recites a process wherein a coating agent consists essentially of cell residue of yeast which has been *solely* treated with enzymes, acidic solution and water to remove internal soluble cell constituents. In claim 70, the recitation "A process *consisting essentially of* ... " excludes steps of enclosing a hydrophobic liquid within the coating material.

By contrast, the Ishiguro patent is directed to a process for generating microcapsules that enclose hydrophobic liquids. See, *e.g.*, the title of the reference ("Process for treating yeast ... to produce microcapsules enclosing hydrophobic liquids") and the Summary of the Invention at col. 2, lines 45-48 ("[T]he present invention provides a process for producing highly practical microcapsules comprising yeast cells in which a hydrophobic liquid is enclosed ..."). Ishiguro clearly does not suggest or disclose a process in which an encapsulated hydrophobic liquid is absent from its microcapsules.

Claim 62

Claim 62 is further distinguished from the methods of Ishiguro, in that this claim recites that the yeast has been pre-treated physically to rupture the cell walls. The instant specification discloses (*e.g.*, at page 10, lines 20-26) that physical rupturing of the cell walls before enzyme treatment facilitates the enzymatic treatment and enhances the formation of coating films having superior physical properties. By contrast, Ishiguro does not suggest or disclose such a pre-treatment, and provides no motivation to modify its method to include such a pre-treatment. If anything, the reference suggests against such a pre-treatment step. See, *e.g.*, col. 4, lines 46-48 of that patent, which disclose that rupture of a microcapsule preparation during encapsulation is undesirable, since such rupture produces "unsatisfactory" capsules. Applicants do not understand the Examiner's allegation in the Office Action of August 26, 2003 that this pre-treatment rupture step, which is neither suggested nor disclosed by the reference, is not a patentable feature of the instant method claims.

Furthermore, it is noted that a Japanese counterpart to the Ishiguro reference (Japanese laid-open Patent Application No. 4-117245) was of record in the prosecution of

the Japanese patent application corresponding to the present application (now registered as JP3349677). That reference was deemed by the Japanese patent examiner not to disclose the invention claimed in that application.

A copy of the published paper Kasai *et al.* (2000), *International Journal of Pharmaceutrics* 204, 53-59, which discusses the preparation of acid-treated yeast cell wall (AYC), is attached.

Ishiguro provides no motivation, with the requisite reasonable expectation of success, to modify its methods to achieve the methods of the instant claims. Therefore, for at least the above arguments, the reference does not render the instant claims obvious (*In re Vaeck*, 20 USPQ2d 1438 (CAFC 1991). The rejection should be withdrawn.

In view of the preceding amendments and arguments, it is believed that the application is in condition for allowance, which action is respectfully requested.

Should any additional fee be deemed due, please charge such fee to our Deposit Account No. 22-0261, and notify the undersigned accordingly.

Respectfully submitted,

Date: May 26, 2004

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DOCS #542456 v2

DECLARATION UNDER 37 C.F.R. 1.132

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Takahide KASAI et al.
Serial No. 09/514,312
Filed: February 28, 2000
For: COATING AGENT

Group Art Unit: 1615

Examiner: Dr. Liliana Di Nola-Baron

Commissioner of Patents and Trademarks
Washington, D.C. 20231

Declaration Under 37 C.F.R. 1.132

Dear Sir:

Takahiro Eguchi declares as follows:

1. I am an inventor of the subject matter of the above-identified patent application.

2. I received a bachelor degree from Kyoto University, where I majored in mechanical engineering, on March 1, 1992.

3. I joined Kirin Brewery Company Limited (Japan) on April 1, 1992, where I have been engaged in research & development in the field of coatings using yeast cell walls for more than 6 years.

4. I have read and understood the Office Action dated August 26, 2003 and U.S. patent 5,521,089 ("Ishiguro"), which was cited therein as the basis for an obviousness rejection. The reference discloses methods of making microcapsules (MC) suitable for encapsulating hydrophobic liquids. In one embodiment, the MC are spread evenly on paper to form pressure-sensitive copy paper. Like the presently claimed methods for making a coating agent, yeast cells are treated in various manners. However, in contrast to the methods of the present application, the reference does not recognize the importance of *not* treating the yeast with alkali. Furthermore, Ishiguro's "coatings" are not disclosed as air-tight, continuous, uniform and non-cracking. As shown below, MC made by a process of the reference in which yeast are treated with alkali do not exhibit such desirable properties, whereas coatings prepared by the claimed method do exhibit such properties.

5. Under my direction and control, cast films were prepared from microcapsules prepared by either of two methods exemplified in the reference or by a method of the invention.

Sample A was prepared by treating yeast with the enzyme, zymolyase 20T, and then treating the digested yeast with alkali, following the protocol of Example 3 in the Ishiguro

reference.

Sample B was prepared by treating yeast with alkali, following the protocol of Example 1 in the Ishiguro reference.

Sample C was prepared according to a method of the present invention, following the protocol of Example 6. The Resulting Acid treated Yeast Cell wall fraction (AYC) dispersed in water to a solids concentration of 8 wt%.

A cast film was made from each of the three samples as follows: each sample was placed on a thin aluminum plate and heated and dried at 120° C for 30 minutes, by means of infrared rays. The resulting films were observed visually, to determine continuity and uniformity.

6. The results of the analysis are shown in Table 1 and in Figures 1-3.

A cast film made with Sample A exhibited many cracks and voids during the drying process. The film was so fragile that its strength could not be measured. The cast film is shown in Figure 1.

A cast film made with Sample B exhibited several cracks. The film was flat and even, but was so fragile that it cracked if it was gently touched. The film was so fragile that it was impossible to attach a test piece of it to the tensile tester in order to measure its strength. The cast film is shown in Figure 2.

A cast film made with Sample C was continuous and uniform, and was non-cracking and flexible. And the film exhibits considerable tensile strength. The cast film is shown in Figure 3.

Table 1

	cracks in the film	strength of the film
MC treated by zymolyase, (prior art reference)	a few cracks many small voids	many small voids too fragile to measure
MC treated with alkali (prior art reference)	several cracks	too fragile to measure
AYC (present invention)	none	Flexible and strong

7. The preceding experiments show a clear difference which would not be expected from the teachings of the Ishiguro reference. The properties of microcapsules made by the methods of the Ishiguro reference in which yeast are exposed to alkali are clearly different from the properties of a coating agent made by the method of the present invention, in which yeast are not exposed to alkali. For example, a film made with the coating agent of the invention exhibits superior film forming ability (forms a continuous, non-cracking film) and acts as a better gas barrier (is air-tight).

8. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-referenced application or any patent issuing thereon.

Date:. . . . . .

. . . . . .

Takahiro Eguchi

DECLARATION UNDER 37 C.F.R. 1.132

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Takahide KASAI et al.
Serial No. 09/514,312 Group Art Unit: 1615
Filed: February 28, 2000
For: COATING AGENT Examiner: Dr. Liliana Di Nola-Baron

Commissioner of Patents and Trademarks
Washington, D.C. 20231

Declaration Under 37 C.F.R. 1.132

Dear Sir:

Takahide Kasai declares as follows:

1. I am a inventor of the subject matter of the above-mentioned patent application.

2. I received a master degree from Osaka University, where I majored in fermentation engineering, on March, 1995.

3. I joined Kirin Brewery Company Limited(Japan) on April 1,1995, where I used to engage in research & development in the field of coatings using yeast cell walls for 3 years.

4. I have read and understood the Office Action dated August 26, 2003 and U.S. patent 5,521,089("Ishiguro"), which was cited therein as the basis for an obviousness rejection. The reference discloses methods of making microcapsules(MC) suitable for encapsulating hydrophobic liquids. In one embodiment, the MC are spread evenly on paper to form pressure-sensitive copy

paper. Like the presently claimed methods for making a coating agent, yeast cells (or yeast cell wall) are treated in various manners. However in contrast to the method of the present application, the reference dose not recognize the importance of ***not*** treating the yeast (or yeast cell wall fraction) ***with alkali.*** Furthermore, in Ishiguro's "coatings", the function of controlling the time at which dissolution of coated materials (ex. medicine) begins are not disclosed. This function is disclosed in the present application of example 5. Especially, the present invention is useful as an enteric coating agent as stated in (2) of page 32.

As, shown below, MC made by a process of the reference in which yeast are treated with alkali do not exhibit such desirable properties, whereas coatings prepared by the claimed method do exhibit such properties. For the person skilled in the art, this phenomenon was completely unobviousness over the Ishiguro reference.

More concretely, the tablet which is coated by the acid treated yeast cell wall fraction has not-releasing time (time lag) in the process of dissolution, but the tablet which is coated by the alkali treated yeast cell wall fraction dose not have not-releasing time (time lag) in the process of dissolution, because of its gradually releasing(sustained-releasing) profile. Therefore, the alkali treated yeast cell wall fraction cannot be used as time-controlled coating which is very useful in the field of drug delivery system (DDS), such as for ***enteric*** coating, and moreover, for masking bitter taste and offensive smell.

5. Under my direction and control, acid treated yeast cell wall fraction and alkali treated yeast cell wall fraction, tablets for dissolution study ,and the coated tablets were prepared in this way.

Fraction2: Acid treated yeast cell wall (This invention)

Acid treated yeast cell wall fraction (**AYC**) that was prepared by means of Example 6 in this applicantion.

Fraction3: alkali treated yeast cell wall (Ishiguro reference)

The yeast cell wall fraction obtained in the form of autolysis yeast residue in Example 1 of this application was treated according to the method described in the Ishiguro reference(Detailed description of preferred embodiments). The concrete method is as follows. The form of autolysis yeast residue in Example 1 of this application meanwhile suspended in 0.1N sodium hydroxide to a solids concentration of 5 wt%, then treated with alkali for 10 minutes at 80℃, and then centrifuged for 15 minutes at 4500rpm to remove the solubilized components, giving an alkali treated yeast cell wall fraction consisting of the resulting residue.

Preparation of core tablets

The formulation of core tablet was shown in Table 1. A mixed powder of AAP (acetaminophen) and lactose was granulated with a fluidized bed (MP-01, Powlex, Osaka) using HPC-L aqueous solution as a binder by the top spray method. The granules obtained were mixed with magnesium stearate and compressed with a rotating tabletting machine (HT-22P HATA, Tokyo) equipped with a 7mm diameter

and 4.5 mm radius of curvature die.

Preparation of coated tablets

The coating agent(fraction 2 or 3) aqueous dispersion containing 5% of coating agent(fraction 2 or 3) and 0.35% of glycerol was used for coating. The coating of core tablets was performed with Driacoater (Powlex, Osaka) at the coating % 25 based on the weight of the core tablet. The operating condition for coating were as follows: core tablets, 250 g; inlet and outlet air temperatures 70 and 45-47℃ respectively.

Table 1

The formulation of core tablet

Acetaminophen (AAP)	3.6
Lactose	112.8
Hydroxypropyl cellulose	3.0
Magnesium state	0.6
Total weigh per tablet	120.0

Release study

The release profiles of AAP from the coated tablet were studied with a dissolution tester (NTR-6100A, Toyama Sangyo, Osaka), according to the paddle method (JP13) using 500 ml of dissolution fluid at 37± 0.5℃ and a rotating paddle at 100rpm. Distilled water was used for the dissolution fluid. The quantity of AAP was determined spectrophotometrically by measuring the absorbance at 242nm.

6. The results of the analysis are shown in Table 2 and Fig1. The tablet that is coated by fraction 3 gradually releases AAP as time goes on, but the tablet that is coated by fraction 2 releases AAP after an initial time lag, and releases AAP quickly after the time lag, that is called sigmoid release curve or sigomoidal profile.

Table 2

	Start time of dissolution	End time of dissolution	Dissolution profile
core tablet	0 min	10 min	-
AYC(This invention) (fraction 2)	60 min	120 min	sigmoidal release
alkali treated yeast cell wall fraction (Ishiguro reference) (fraction 3)	0 min	1440 min	gradual release (sustained-release)

Note Base⇒ Alkali treated yeast cell wall fraction= alkali treated yeast cell wall fraction

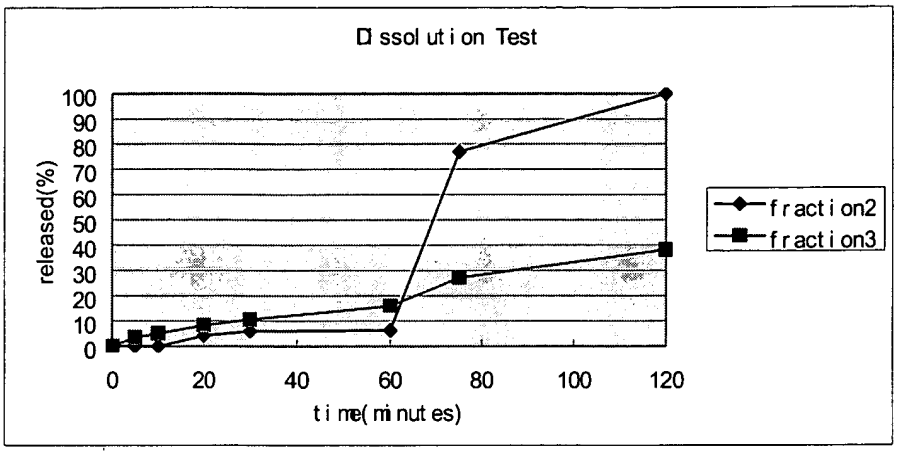

Fig. 1

7. The preceding experiments show a clear difference which would not be expected from the teachings of Ishiguro reference, and shows that the alkali treatment for yeast cell wall makes the profile of gradually releasing(sustained-release) profile, but not-alkali treatment for yeast cell wall makes the profile of sigmoidal profile.

Reportedly, the sigmoidal release profiles are obtained by *blending the polymers of different kinds or adding other materials such as a swelling agent or an organic acid* (Narisawa et al., 1996[1],1997[2]) but, the sigmoidal release profile was obtained by coating agent; ***the AYC aqueous dispersion*** of this invention.

8. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above referenced application or any patent issuing thereon.

1) Narisawa, S., Nagata, M., Hirasawa, Y., Kobayashi, M., Yoshino, H., 1996
An organic acid-induced sigmoidal release system for oral controllede-release preparations 2 Permeability enhancement of Eudragit RS coating led by the physicochemical interactions with organic acid J. Pharm. Sci. 85, 184-188

2) Narisawa, S., Nagata, M., Hirasawa, Y., Kobayashi, M., Yoshino, H., 1996
An organic acid-induced sigmoidal release system for oral controlled-release preparations III Elucidation of the anomalous drug release behavior through osmotic pumping mechanism. Int. J. Pharm. 148, 85-91.

Date:.....

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Takahide Kasai

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Application of acid-treated yeast cell wall (AYC) as a pharmaceutical additive I. AYC as a novel coating material

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Abstract

Acid-treated yeast cell wall (AYC) was newly prepared by acidifying the cell wall of brewer's yeast and the potential to use AYC as a novel coating material was studied. AYC had an oval shape with the diameter of several μm. The rheogram of AYC aqueous dispersion showed the plastic fluid property that is generally observed in the suspension. Core tablets containing 3% of acetaminophen (AAP) were coated with the AYC aqueous dispersion containing 5% (w/v) of AYC and 0.35% (w/v) of glycerol at various coating percents. The AAP release profile from the AYC-coated tablets was studied by the JP13 paddle method using solutions at various pH. Tensile strength and permeability of oxygen and water vapor of AYC cast film were measured. The AAP release from the AYC-coated tablets showed sigmoidal release profile with an initial lag time and the duration of the lag time depended on the coating percent of AYC. The pH of the dissolution fluid or the storage at room temperature for 120 days had little affect on AAP release from the AYC-coated tablets. These results suggest that it is possible to control the start time of medicine release independent of the pH by coating of AYC, that is the time-controlled release. The AYC cast film showed a large tensile strength and an extremely small oxygen permeability coefficient and a sufficient level of water permeability coefficient in order to protect from moisture. These results present that AYC has the high utility as a novel aqueous coating material for DDS preparations.

Keywords: Yeast; Coating material; Lag time; Sigmoidal release; pH; Oxygen permeability

1. Introduction

Synthetic polymers such as cellulose, methacrylic acid copolymers or polyvinyl polymers are generally used for film coating of phar-

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maceuticals. Recently, however, natural materials have been attended from the viewpoint of utilization of available resources and safety. Reportedly, sodium alginate (Kaneko et al., 1999), xanthan gum (Watanabe et al., 1991), locust bean gum (Watanabe et al., 1991), chitosan (Hou et al., 1991) and pectin (Ashford et al., 1994) have been attempted for use as natural coating materials. In this study, as well as these polymers, we noticed beer yeast cell wall as a natural material for coating.

Yeast is a micro-organism, which has been used for brewing and baking since ancient times, but recently applications based on its physiological, metabolic or nutrient-rich characteristics have appeared in food and pharmaceutical industries. These applications, however, mainly utilized the characteristics and components of the yeast cytoplasm.

In the series of this study, in order to find a new and effective use for yeast cell wall, we have prepared the acid-treated yeast cell wall (AYC) and developed the application of AYC to pharmaceutical additives. In this report, we attempted to apply AYC as a novel coating material and investigated the possibility of actual coating of AYC and the functions of the AYC-coated layer.

2. Materials and methods

2.1. Materials

Brewer's yeast (*Saccharomyces cerevisie*, Kirin Brewery, Tokyo) collected after the manufacturing of beer was used as a raw material. Acetaminophen (AAP, Tokyo Kasei Kogyo, Tokyo) was used as a model drug. Hydroxypropylcellulose (HPC-L, viscosity 6.0–10.0 cps, Shin-Etsu Chemical, Tokyo), magnesium stearate (Wako Pure Chemical Industries, Osaka), glycerol (Wako Pure Chemical Industries, Osaka) and Lactose (DMV Japan, Tokyo) were used as a binder, lubricant, plasticizer and excipient, respectively.

2.2. Preparation of AYC

Manufacturing of AYC was performed through an acid treatment process because lysinoalanine, which is noxious for the human body, was generated by heat alkali treatment. Intracellular components of the intact yeast were solubilized by reaction with intracellular or external enzyme, such as proteases and glucanases, and the soluble components were removed. The acidifying reaction was then carried out with 5% (w/v) of the residual fraction and 0.5 N HCl at 80°C for 20 min. After centrifugation, the precipitates were thoroughly washed with water. The pH of the system was adjusted to 9.0 in order to remove the bitterness substances from hops as the brewer's yeast used in this study was actually the residue used from the manufacture of beer. The pH was adjusted to 3.8–4.2 and the AYC was then obtained after centrifugation and washing with water.

2.3. Observation of surface of yeast and AYC

The surface of the brewer's yeast and AYC was observed by scanning electron microscopy (SEM) using an ultra-high revolution, low-velocity scanning electron microscope (UHR-SV SEM/ S-900LV 1KV, Hitachi, Tokyo).

2.4. Analysis of AYC components

The protein content of the AYC was analyzed by Kjeldahl method (Nakajima et al., 1988) and the lipid content was measured using the method described by Folch et al. (Folch et al., 1957). The crude fiber content was analyzed by the standing method (Sample Analysis Standard Workshop, 1998) and the mineral content was analyzed by the direct ashing method. The amount of nitrogen free extract was calculated by subtracting the content of the protein, lipid, crude fiber and ash from the total content.

2.5. Dispersion state and rheological properties of AYC aqueous dispersion

An AYC aqueous dispersion containing 6% of

AYC was centrifuged at 5000 rpm for 5 min and shaken by hand for several seconds to disperse AYC again. The dispersion state of AYC before and after shaking was observed. The rheological property of the AYC aqueous dispersion was evaluated from the relationship between shearing-rate and shearing-stress using a corn-plate viscometer (Brookfield Engineering Laboratories, a digital viscometer model DV-II+) at various concentrations of AYC.

2.6. Preparation of core tablets

The formulation of the core tablet was shown in Table 1. A mixed powder of AAP and lactose was granulated with a fluidized bed (MP-01, Powlex, Osaka) using HPC-L aqueous solution as a binder by the top spray method. The granules obtained were mixed with magnesium stearate and compressed with a rotating tabletting machine (HT-22P HATA, Tokyo) equipped with a 7 mm diameter and 4.5 mm radius of curvature die.

2.7. Preparation of AYC-coated tablets and measurement of thickness of AYC-coated layer

The AYC aqueous dispersion containing 5% of AYC and 0.35% of glycerol was used for coating. The coating of the core tablets was performed with Driacoater (Powlex, Osaka) at the coating % of 10, 25, 43 and 67 based on the weight of the core tablet. The operating conditions for coating were as follows: core tablets, 250 g; inlet and outlet air temperatures, 70 and 45–47°C respectively; air volume, 0.83 m^3/min; spray pressure, 1.5 kgf/cm^2; spray rate, 5–7 g/min; spray air volume, 28 l/min; pan revolution, 30 rpm; curing temperature, 80°C; curing time, 90 min. The thickness of the AYC-coated tablet was measured with a dial thickness gauge (Mitsutoyo, Tokyo). The thickness was determined as the distance between the top of the curvature of the AYC-coated tablet. The thickness of AYC-coated layer was calculated by subtracting the thickness of the core tablet from that of the AYC-coated tablet.

Table 1
Formulation of core tablet (mg)

Acetaminophen (AAP)	3.6
Lactose	112.8
Hydroxypropyl cellulose	3.0
Magnesium sterate	0.6
Total weight per tablet	120.0

2.8. Release study

The release profiles of AAP from the AYC-coated tablet were studied with a dissolution tester (NTR-6100A, Toyama Sangyo, Osaka), according to the paddle method (JP13) using 500 ml of dissolution fluid at 37 ± 0.5°C and a rotating paddle at 100 rpm. Distilled water (pH 5.8), buffer solutions composed of NaCl and HCl of pH 1.2, CH_3COOH and CH_3COONa of pH 4.0. CH_3COOH and CH_3COONa of pH5. 0, KH_2PO_4 and Na_2HPO_4 of pH 6.0, KH_2PO_4 and NaOH of pH 7.0 and $CH_2(NH_2)CH_2OH$ and HCl of pH 8.0 were used for the dissolution fluid. The quantity of AAP was determined spectrophotometrically by measuring the absorbance at 242 nm.

The apparent change of the AYC-coated tablet during the release process was observed with optical microscope (Nikomat, Nikon, Tokyo).

2.9. Preparation of AYC cast films

The AYC cast film was prepared with the AYC aqueous dispersion containing 3.5% of AYC and 0.35% of glycerol. After degassing, the dispersion containing 1 g of AYC was placed in a plastic plate with the dimensions of 9.0 × 13.0 cm and drying at 40°C for 24 h. The AYC cast film obtained was dark brown and translucent. The thickness of the AYC cast film was about 60 μm.

2.10. Measurement of tensile strength and oxygen and water vapor permeability coefficients of AYC cast film

The tensile strength of the AYC cast film cut in the dumbbell shape was measured by the JIS Z1702 method with a universal testing machine (CATY1001-ZS, Yonekura Seisakusho, Osaka) at

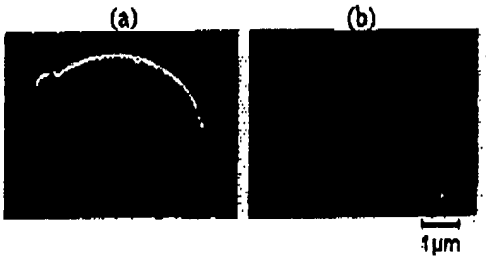

Fig. 1. SEM photographs of crude brewer's yeast (a) and AYC (b).

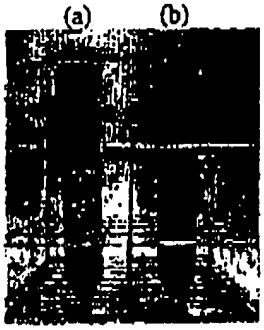

Fig. 2. Appearance of AYC aqueous dispersion. (a), centrifuged at 5000 rpm for 5 min; (b), after shaking (a) by the hand.

23°C, 50% RH and 50 mm/min of a cross-head speed. The oxygen permeability coefficient of the AYC cast film was measured by JIS K7126B method with a TRAN10/50 (MOCON, Minneapolis) at 23°C and 0% RH. The area of AYC cast film tested was 50 cm^2 and oxygen concentration was 100%. The water vapor permeability coefficient of the AYC cast film was measured by JIS Z0208 method with a PL4SP incubator (Tabaiesupekku, Osaka) and balance (AE200, Mettler-Toledo, Greifensee) at 40°C and 50% RH. The area of AYC cast film tested was 28.26 cm^2.

3. Results and discussion

3.1. Shape and components of AYC

SEM photographs of crude brewer's yeast and AYC are shown in Fig. 1(a) and (b), respectively. Brewer's yeast is classified as Ascomycetes and has an oval shape with the diameter of about 6–10 μm. By comparing these two photographs it can be seen that the size of crude brewer's yeast and AYC are almost the same. The crude yeast has a smooth surface, but AYC has a rough surface and a distorted shape.

The chemical components of the yeast cell wall are different depending on the species. In the case of *S. cerevisiae* (known as a brewer's yeast and a baker's yeast) is composed of mainly polysaccharides such as glucan and mannan and a little protein (Northcote and Horne, 1952; Fleet and Manners, 1976). A double-layer model is advocated for the structure of the yeast cell wall, composed of a mannan-protein complex as the upper layer and of glucan as the lower layer (Lampen, 1968; Kidby and Davies, 1970). Therefore, the change in the surface and shape may be due to loss of mannan and protein of the yeast outer layer during the acidifying process.

The component of AYC was as follows: nitrogen free extract content, 58%; crude fiber, 30%; protein, 10%; lipid, 0.3% and ash, 0.7%. Dietary fiber is generally defined as the nitrogen-free extract and crude fiber. So, dietary fiber content was estimated as 88%.

3.2. Dispersion state and rheological properties of AYC aqueous dispersion

Fig. 2 (a) shows the AYC aqueous dispersion containing 6% of AYC after centrifugation at 5000 rpm for 5 min. It was observed that the cake of AYC had settled at bottom of the centrifuge tube. When this was shaken gently by the hand for several seconds, AYC was homogeneously re-dispersed (b) and the dispersion state was maintained for at least 6 h. This may be due to only a slight difference in the apparent density between water and sufficiently water-absorbed AYC.

Fig. 3 shows the rheogram of the AYC aqueous dispersion at various concentrations of AYC. All dispersions have the yield value and a linear relationship was observed between the shearing-stress and the shearing-rate without hysteresis, indicating a plastic fluid property. Polymer solutions

generally used for coating show the quasi-viscous fluid property without the yield value (Martin et al., 1983; Ichibagase et al., 1997). This is because of the mutual folding and tangling of the linear polymer molecules at low shearing-stress is reduced with the increased shearing-stress, and then the polymer molecules align in the direction of flow. In contrast, AYC was insoluble to water and AYC aqueous dispersion was a suspension that AYC swollen particles dispersed in. Therefore, the plastic flow property was observed.

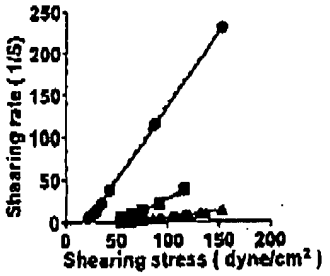

Fig. 3. Rheogram of AYC aqueous dispersion at various AYC concentrations. AYC concentration: ●, 4%; ■, 5%; ▲, 6%. Full line: increase in shearing-stress. Broken line: decrease in shearing-stress.

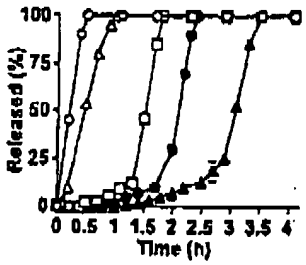

Fig. 4. Effect of AYC coating percent on AAP release from AYC-coated tablets. Coating %; ○, 0 (core tablet); △, 10; □, 25; ●, 43; ▲, 67. Each point represents the mean ± S.D. ($n = 3$).

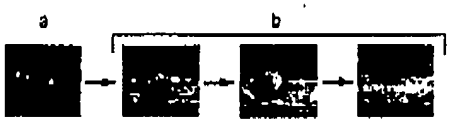

Fig. 5. Apparent change of AYC-coated tablet during release process. a. before the dissolution test: b. during the dissolution test.

It was found that AYC was dispersed as an independent particle in water unlike other polymers generally used as a solution for the film coating, and the dispersion state of AYC aqueous dispersion was maintained for a long time. These results suggest that the AYC aqueous dispersion is useful for the actual coating without the necessity for the agitation during the coating process.

3.3. AAP release profile from core tablet and AYC-coated tablets at various coating percents of AYC

Fig. 4 shows AAP release profiles from the core tablet and the AYC-coated tables at various AYC coating % in the distilled water. The thickness of the AYC-coated layer at coating percents of 10, 25, 43 and 67 were about 210, 470, 710 and 1140 μm, respectively. Rapid release of AAP from the core tablet was observed. In contract, the AAP release from the AYC-coated tablets showed sigmoidal release profile with an initial lag time and the duration of the lag time depended on the coating percent of AYC. This result indicates that AYC film is certainly formed on the surface of the core tablet and functions as a coating layer, which makes the release a sigmoidal profile with a lag time. This sigmoidal profile with an initial lag time may be useful for masking bitter taste and offensive smell (Shirai et al., 1993; Kaneko et al., 1997; Sugao et al., 1998). Reportedly, the sigmoidal release profiles are obtained by blending the polymers of different kinds or adding other materials such as a swelling agent or an organic acid (Narisawa et al., 1994; Ueda et al., 1994a,b; Yamakita et al., 1996; Narisawa et al., 1996, 1997). In this study, the sigmoidal release profile was obtained by coating only the AYC aqueous dispersion.

Slight difference in the release rate of AAP after the lag time was observed. This result is caused by the fact that AAP release from the AYC-coated tablet began with a collapse of the AYC-coated layer as shown in Fig. 5, although the drug is released by the dissolution of the coating film in the case of other coating materials. The increase in the coating percent of AYC caused the increase in the thickness of the AYC-coated layer. There-

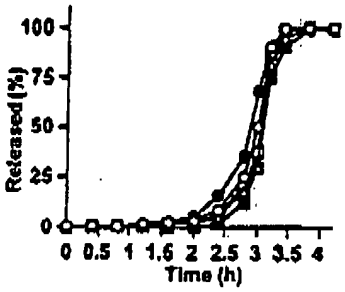

Fig. 6. Effect of pH of dissolution fluid on AAP release from AYC-coated tablets. pH: ○, 1.2; △, 4.0; □, 5.0; ●, 6.0; ▲, 7.0; ■, 8.0.

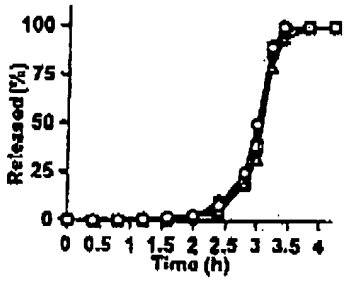

Fig. 7. Effect of storage period on AAP release profiles from AYC-coated tablets. Storage period (d): ○, 0; △, 30; □, 60; ●, 90; ▲, 120.

Table 2
Tensile strength and oxygen and water vapor permeability coefficients for AYC cast films

Tensile strength (Mpa)	39.6 ± 2.5^a
Oxygen ($cm^3 \cdot mm/m^2 \cdot 24\ h \cdot atm$)	$5.0 \times 10^{-3} \pm 1.6 \times 10^{-3a}$
Water vapor ($g \cdot mm/m^2 \cdot 24\ h$)	17.1 ± 0.6^a

[a] The values represent the mean ± SD ($n = 5$)

fore, the extension in the lag time with the increasing coating percents of AYC may be due to the increase in the strength of the AYC-coated layer and the decrease in the permeation rate of the dissolution fluid.

3.4. Effects of pH of dissolution fluid and storage period on AAP release

Figs. 6 and 7 show the effects of the pH of dissolution fluid and the storage period on AAP release from the AYC-coated tablets, respectively. The AYC coating percent was 67. The AYC-coated tablets had been stored at room temperature under 50% RH. The pH and the storage for 120 days had little affect on AAP release from the AYC-coated tablets. These results indicate that the coating of AYC can make the time-controlled release system independent of pH of the dissolution fluid.

3.5. Tensile strength and permeability coefficients of oxygen and water vapor into AYC cast film

The tensile strength and the oxygen and water vapor permeability coefficients for the AYC cast films are listed in Table 2. The tensile strength was 39.6 ± 2.5 MPa (e.g. The values of hydroxypropylmethylcellulose (HPMC) and HPC are 43.0 and 43.2 MPa, respectively, which are estimated from the product brochures of Shin-Etsu Chemical and Nippon Soda). The oxygen permeability coefficient was an extremely small value, as it was equal to the value of the aluminum foil laminated with polyethylene and polyethyleneterephthalate (1.8×10^{-3} $cm^3 \cdot mm/m^2 \cdot 24\ h \cdot atm$, Yoshii and Yamaguchi, 1998). The water vapor permeability coefficient was a sufficient level in order to protect it from moisture (e.g. The values of HPMC and HPC are 81.0 and 168.6 $g \cdot mm/m^2 \cdot 24$ h, respectively, which are estimated from the product brochures of Shin-Etsu Chemical and Nippon Soda).

4. Conclusion

The sigmoidal release profile with an initial lag time was obtained by coating the AYC aqueous dispersion and the release profile was hardly affected by pH or the storage period for 120 days. The AYC cast film had an extremely low oxygen permeability and a sufficient water vapor permeability level in order to protect it from moisture. Our results suggest that the AYC has a high potential utility as a novel aqueous coating material for DDS preparations.

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